# Addendum to DeCyder 2D™ 7.0 User Manual, 28-9414-47 AA

## Introduction

DeCyder 2D version 7.1 is an upgrade from 7.0 with a new functionality to normalize abundance values using spike proteins. This is a complement to the present model based normalization. It is suitable for experiments where one or several proteins have the same presence in all samples and can be used as references for the normalization. The spike normalization is added in the DIA and the BVA modules.

## Spike Normalization

### Limitations of model based spot normalization

The model based spot normalization in DeCyder 2D DIA and BVA is based on the assumption that the majority of all proteins in a gel keep their expression level between the different samples in an experiment. In a classic DIGE experiment it is important that at least 100 spots, preferably 2000-3000 spots, are detected to get a valid normalization. However, this approach is not optimal for applications with samples that have few protein spots or samples where the majority of the proteins differ in expression level between samples.

### Summary and benefits of spike normalization

To expand the usability of the software, an alternative normalization tool based on so called spiked proteins is introduced in DeCyder 2D 7.1. With this it is possible to normalize the proteins in an experiment to user selected proteins in the samples. Spike proteins must be added to the samples prior to the CyDye labelling step. It is important to select spike proteins with a minimum of interference/overlay with any protein spots in the sample 2D map. They should also be of other origin/species than the sample proteins to avoid interference with the differential analysis or protein identification errors. So called housekeeping proteins of the sample itself (proteins known to be of constant concentration in all samples) can also be used for the spike normalization procedure.

A typical application where the spike normalization is applicable is for the accurate determination of host cell proteins (HCP) in the downstream purification process for biopharmaceutical products.

## Detailed description of spike normalization

Spike normalization is an alternative to the model based normalization. It still normalizes volume ratios but based on a few spike proteins and not the complete set of proteins in a whole workspace.

The model based normalization is described in Appendix B.2 "*Detailed description of spot normalization procedure*" in *DeCyder 2D 7.0 User Manual*.

### *Spike normalization in DIA module*

In the DIA module, spike normalization will adjust the volume ratio so the mean volume ratio for all spike proteins will be the 1.0 volume ratio. The spike volume ratio is calculated as follows:

$R_i = (V_{1i}/V_{2i}) / R_{spike}$

Where $R_i$ is the spiked volume ratio for the i:th protein

$V_{1i}$ is the spot volume in one sample for the i:th protein

$V_{2i}$ is the spot volume in the other sample for the i:th protein

$R_{spike}$ is the raw mean volume ratio for all spike proteins

### *Spike normalization in BVA module*

In the BVA module, spike normalization will normalize the standard abundance value for all proteins so the mean value of all spike proteins standard abundance will be set to 1.0. The spike standard abundance is calculated as follows:

$S_i = (V_{ix}/V_{ixs}) / S_{spike}$

Where $S_i$ is the spiked standard abundance for the i:th protein

$V_{ix}$ is the spot volume for the i:th protein and the x:th spot map

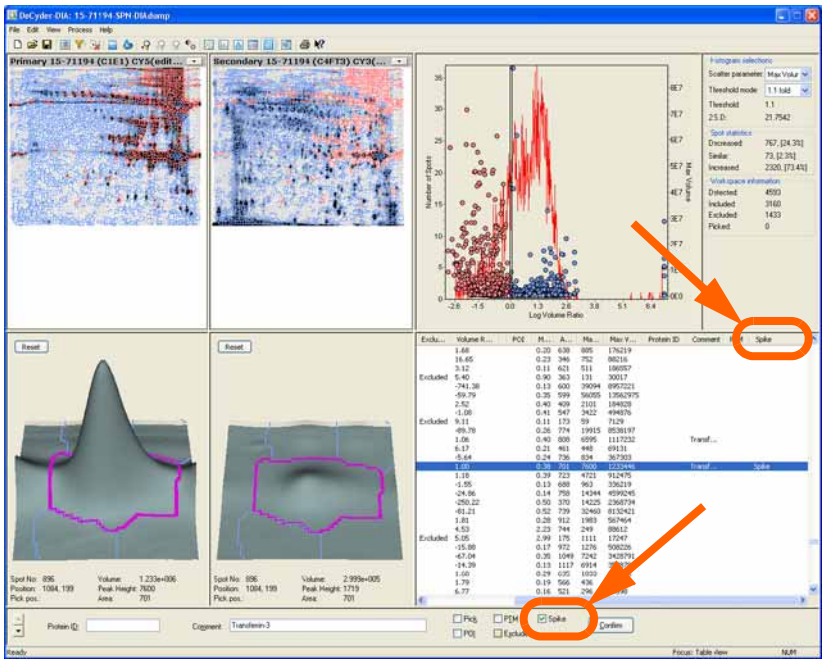
$V_{ixs}$ is the spot volume for the standard spot corresponding to the i:th protein and the x:th spot map

$S_{spike}$ is the mean standard volume ratio for all spike proteins

# Details of new features in DIA module

In the DIA Main window, there are two visible changes in version 7.1:

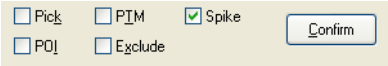
- in the Spot Control Panel there is a new checkbox, **Spike**.
- in the table view there is a new column, **Spike**. The word **Spike** in the new column indicates that the protein is assigned as a spike protein.



When spike normalization will be used in the BVA module, perform all normalization in the BVA module. Spike normalization performed in the DIA module will not be included when importing the workspace into the BVA module.

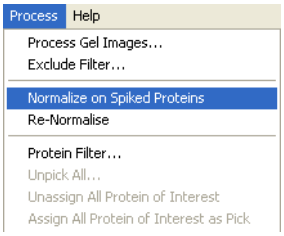
## How to use spike normalization in DIA

1 Identify the spiked protein(s) using the gel view. Select the spike proteins one by one and tick the **Spike** checkbox to indicate that this is a spiked protein.




***Tip:*** *Use the comment field for the spiked protein name to have better control and be able to vary the assigned spiked proteins.*

2 When all spiked proteins are assigned, click on the **Process** menu and select **Normalize on Spiked Proteins** to perform the spike normalization.




3 Verify the normalization by viewing the volume ratios for the spiked proteins one by one. They should all have volume ratios close to 1.0 or -1.0. This is also shown in the histogram scatter plot but the values are close to 0.0 ( = log(1.0)). If a single spike protein is used the volume ratio is 1.0 and the protein is placed on 0.0 in the histogram scatter plot.

The result of the normalization is displayed in the Histogram View, with histograms showing the volume ratios. If there is only one spike protein, it will be in the middle of the graph at point 0.0 (i.e. log(1.0) ). Compared to the model based normalization the pattern in the graph will remain exactly the same but shifted to its spike normalized values.

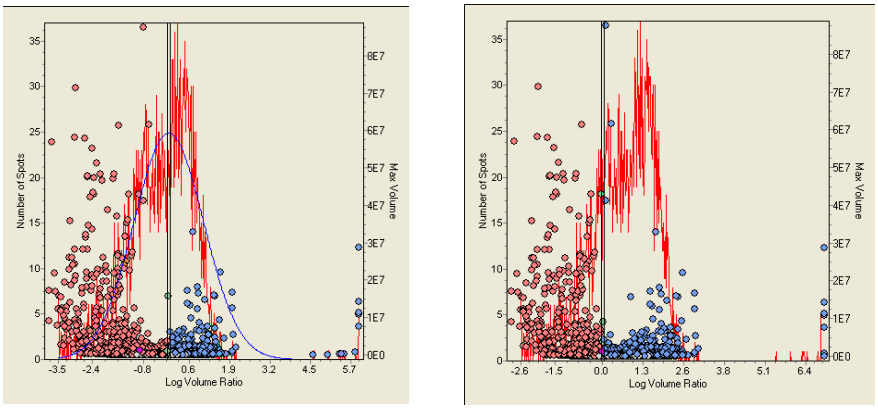


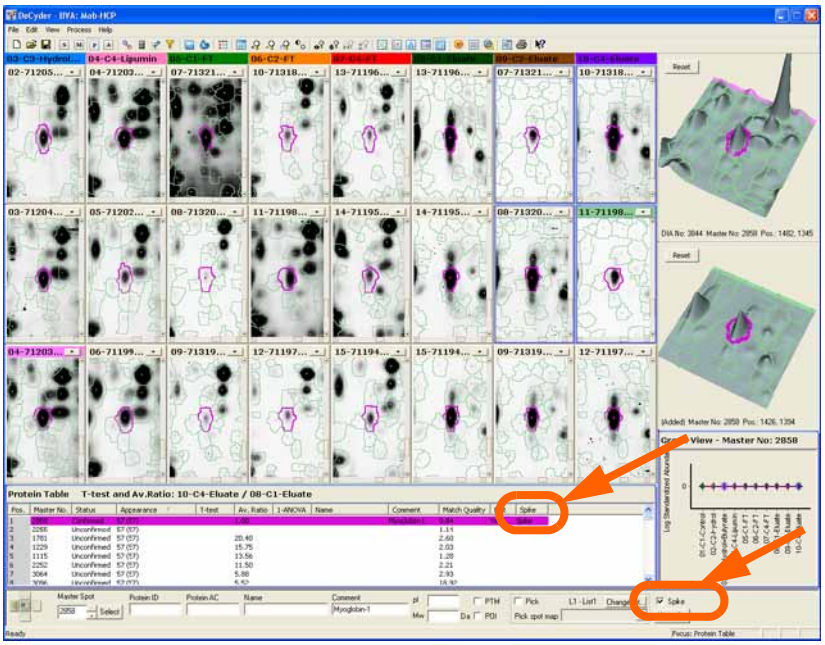

**Figure 1.** The histogram graph for a spike protein when normalized with model based normalization (left) and spike normalization (right). Note that the plot of volume ratios has the same pattern for both types of normalization but is shifted to the right for the spike normalization.

***Note:*** *The menu item **Process->Re-normalize** is the same as before and will force a recalculation of the selected normalization. Normally this is done automatically when changing the spike assignments but not when changing excluded spots.*

# Details of new features in BVA module

In the BVA Main window, there are two visible changes in version 7.1:

- In the Data View Control Panel there is a new checkbox, **Spike**.
- In the Protein table there is a new column, **Spike**. The word **Spike** in the new column indicates that the protein is assigned as a spike protein.



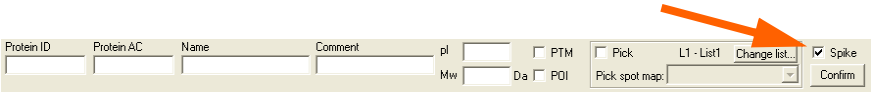
When spike normalization will be used in the BVA module, perform all normalization in the BVA module. Spike normalization performed in the DIA module will not be included when importing the workspace into the BVA module.

## How to use spike normalization in BVA

1 Identify the spiked protein(s) using the gel view in **P** (Protein Mode). Check the appearance number for all spiked proteins. For a good normalization all spiked proteins should be present in all spot maps.

If a spike protein is mis-matched or missing in a spot map, go to **M** (Match Mode). Use the spot editing options in the Data View Controls to correct the matching until all spike proteins are present and matched in all spot maps. (See 5.9.2 Available editing options in *DeCyder 2D User Manual*.)

2 Go to **P** and select the spiked proteins one by one and tick the **Spike** checkbox to indicate that it is a spiked protein.




***Tip:*** *Use the comment field for the spiked protein name to have better control and be able to vary the assigned spiked proteins.*

3 When all spiked proteins are assigned, click on the **Process** menu and select **Normalize on Spiked Proteins**, to perform the spike normalization.

4 Verify the normalization by viewing the standard corrected abundance for the spiked proteins, one by one. They should all have volume ratios close to 1.0 or -1.0. This is also shown in the graph, the values should be close to 0.0 ( = log(1.0) ). The line between the experimental groups is close to horizontal for all spiked proteins. If a single spiked protein is used the volume ratio is 1.0 and the line is horizontal.

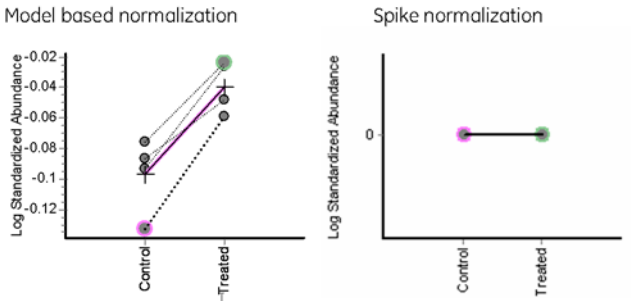



**Figure 2.** The graph for a spike protein when normalized with model based normalization (left) and spike normalization (right). All spike proteins should have a graph between their experimental groups close to the 0.0 point (i.e. log(1.0) ).

## Installation of DeCyder 2D version 7.1

The installation guide can be found in the DeCyder installation via the start menu.

The procedure for upgrading is a bit different depending on whether DeCyder 2D 6.5 or 7.0 is already installed:

- If DeCyder 2D 6.5 is installed:
  Backup the DeCyder database and then uninstall the complete software package (see Section 5 "*Uninstallation*" in the "*DeCyderInstallationGuide.pdf*") before installing.
- If DeCyder 2D 7.0 is installed:
  Backup the DeCyder database and then uninstall the database and the program part (see Section 5 "*Uninstallation*" in the "*DeCyderInstallationGuide.pdf*") before installing. The Oracle Server can remain installed.

## Licensing

Access to the spike normalization is controlled by the eLicense system. For details on eLicensing, see Section 4, "*eLicense installation and activation*", in the "*DeCyderInstallationGuide.pdf*". The installation guide can be found in the DeCyder installation via the start menu.

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/decyder



GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation
Sanken Bldg.3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

imagination at work

28-9680-43 AA 01/2010